

HUMAN INTERLEUKIN-17 RECEPTOR LIKE MOLECULE

FIELD OF INVENTION

The application relates to a newly identified polynucleotide, polypeptide encoded by such polynucleotide, the use of such polynucleotide and polypeptide, as well as the production of such polynucleotide and polypeptide. More particularly, the polypeptide of the present invention is human interleukin-17 receptor like molecule (IL-17RLM).

BACKGROUND

IL-17 is a T cell-derived cytokine that plays an important role in the initiation or maintenance of the proinflammatory response(1-5). Recently, four new proteins are identified and termed as IL-17B, IL-17C, IL-17E/IL-25 and IL-17F/ML-1/IL-26 that are clearly related to IL-17, suggesting that there exists a family of IL-17-like molecules (6-13).

Three homologous receptors for IL-17 family members are also identified, termed as IL-17 receptor, IL-17BR (also known as IL-17Rh1) and IL-17RL (14). IL-17 receptor (IL-17AR) located on human chromosome 22q11.22-11.23 is widely expressed in different tissues and is reported to bind to IL-17A with a weaker affinity than the potency of IL-17A on responsive cells. This receptor is shown to regulate the activities of extracellular regulated kinase ERK1, ERK2, c-Jun N-terminal kinase (JNK), p38 mitogen-activated protein kinase, Raf-1 kinase, STATs and NF- κ B (15-20). Furthermore, the recent report that IL-17AR signaling is deficient in TRAF-6-deficient cells strongly suggests that members of the TRAF family, known to be involved in both IL-1/Toll and TNF receptor signaling, are also involved in IL-17AR signaling (21). The proinflammatory function and intracellular signaling pathway of IL-17AR are strikingly similar to those of the IL-1 and Toll receptors (22-26). IL-17BR (IL-17Rh1) located on human chromosome 3p21.1 is expressed mostly in liver and kidney tissues. This receptor binds to IL-17B and IL-17E but not IL-17A (8). Moreover this receptor is shown to activate NF- κ B only by luciferase assay in vitro. IL-17RL located on human chromosome 3p25.3-3p24.1 is expressed mainly in prostate, cartilage, kidney, liver, heart, and muscle tissues (14), which has at least eleven splicing forms. However, the signaling mechanism and the biological functions of this receptor are still unknown.

Thus so, in an attempt to identify new IL-17 receptor like membrane proteins, the inventors have isolated and identified a novel single span transmembrane type I cytokine receptor-like protein with 31% amino acid identity to IL-17 receptor, and designated the new receptor as hIL-17RLM (IL-17 receptor like molecule).

SUMMARY OF THE INVENTION

In accordance with one aspect of the present invention, there is provided a novel polypeptide, as well as biologically active and diagnostically or therapeutically useful fragments thereof. The polypeptide of the invention is of human origin and designated as hIL-17RLM. hIL-17RLM has two alternative splicing forms, namely hIL-17RLM-L and hIL-17RLM-S, with an amino acid sequence of SEQ ID NO:2 and SEQ ID NO:4, respectively. hIL-17RLM-L shares a 75% amino acid sequence identity with hIL-17RLM-S.

In accordance with another aspect of the invention, there is provided an isolated polynucleotide encoding a polypeptide of the invention including mRNA, DNA, cDNA, genomic DNA as well as biologically active and diagnostically or therapeutically useful fragments thereof. Preferably, the polynucleotides encoding the two splicing forms of the hIL-17RLM of the invention, hIL-17RLM-L and hIL-17RLM-S, have the nucleotide sequence of SEQ ID NO:1 and SEQ ID NO:3, respectively.

In accordance with yet a further aspect of the present invention, there is provided a process for producing such polypeptide by recombinant techniques comprising culturing recombinant prokaryotic and/or eukaryotic host cells containing a nucleic acid sequence encoding a polypeptide of the present invention, under conditions promoting expression of said polypeptide and subsequent recovery of said polypeptide.

In accordance with yet a further aspect of the present invention, there is provided a process for utilizing such polypeptide, or polynucleotide encoding such polypeptide for therapeutic purposes. It is expected that the novel protein would be one promising therapeutic target in some diseases or carcinoma, such as rheumatoid arthritis, asthma, kidney or testis related carcinoma, and neuron diseases.

In accordance with yet a further aspect of the present invention, there are provided antibodies against such polypeptides.

In accordance with still another aspect of the present invention, there is provided nucleic acid probes comprising nucleic acid molecules of sufficient length to specifically hybridize to a nucleic acid sequence of the present invention.

In accordance with still another aspect of the present invention, there is provided a method for treating an individual in need of an increased level of IL-17RLM polypeptide activity in the body comprising administering to such an individual a composition comprising a therapeutically effective amount of an isolated IL-17RLM polypeptide of the invention or an agonist thereof.

In accordance with still another aspect of the present invention, there is provided a method for treating an individual in need of a decreased level of IL-17RLM polypeptide activity in the body comprising administering to such an individual a composition comprising a therapeutically effective amount of an IL-17RLM antagonist.

These and other aspects of the present invention should be apparent to those skilled in the art from the teachings herein.

BRIEF INTRODUCTION OF DRAWINGS

The following drawings are illustrative of embodiments of the invention and are not meant to limit the scope of the invention as encompassed by the claims.

FIG. 1. Sequence information of IL-17RLM, in which:

- A. Nucleotide and deduced amino acid sequence of interleukin-17 receptor like molecule(hIL-17RLM-L); B. Sequence alignment of IL-17RLM alternative splicing forms; C. Sequence alignment of human IL-17RLM and human IL-17 receptor; D. Genomic structure of human IL-17RLM

FIG. 2. Expression of IL-17RLM, in which:

- A. Tissue distribution of IL-17RLM mRNA; B. RT-PCR analysis for IL-17RLM expression; C. Endogenous expression of IL-17RLM protein in mouse tissues; D. Subcellular localization of the IL-17RLM protein; E. Immunohistochemical detection of IL-17RLM protein in various tissues.

FIG 3. The activation of Stat5 signal pathway by IL-17RLM, in which:

- (A) EPOR/hIL-17RLM chimeric receptor promotes STAT5 transcription activity; (B) EPOR/hIL-17RLM chimeric receptor mediates STAT5 tyrosine phosphorylation; (C) STAT5 DNA-binding activity was mediated by EPOR/IL-17RLM chimeric receptor upon stimulation of EPO; (D) EPOR/IL-17RLM chimeric receptor mediates STAT5 DNA-binding activity in a time course manner; (E) EPOR/IL-17RLM chimeric receptor mediates STAT5b DNA-binding activity.

FIG 4. Homodimerization of EPOR/IL-17RLM leads to the proliferation of Ba/F3 cells, in which

FIG. 4A shows that IL-17RLM cytoplasmic domain can transduce a proliferation signal in Ba/F3 cells in a dose dependent manner; and FIG. 4B shows the time course of proliferation.

FIG 5. Interaction of hIL-17RLM-L with mFGFR, in which:

- A. Co-immunoprecipitation of hIL-17RLM-L and mFGFR1; B. Co-immunoprecipitation of hIL-17RLM-L and mFGFR2; C. Endogenous colocalization of IL-17RLM with FGFR1 in some tissues; and D. Colocalization of IL-17RLM with FGFR2 in transfected Cos7 cells

FIG 6. Effects of hIL-17RLM-L on differentiation of PC12 cells, in which:

A and B, Stably expressed hIL-17RLM-L significantly inhibits FGF2 or NGF-induced PC12 cell differentiation; C and D, hIL-17RLM-L strongly inhibits FGF2 or NGF-induced PC12 cell differentiation even in higher stimulation or elongated exposure of FGF2 or NGF; E and F, The intracellular domain of IL-17RLM-L is the functional domain for the negative effect of hIL-17RLM-L on PC12 cell differentiation.

FIG 7. Effects of hIL-17RLM-L on Ras-MAPK signal pathway during bFGF-induced differentiation of PC12 cells, in which:

A. The intracellular domain of IL-17RLM is the functional domain for the inhibitory effects of hIL-17RLM-L on Ras-MAPK signal pathway; B, C. Dose dependent effects of hIL-17RLM-L(WT) and N-terminal truncated hIL-17RLM-L (ΔN) on Ras-MAPK signal pathway by luciferase assay; D, E. hIL-17RLM-L inhibits FGF2-induced ERK activation in PC12 cells by Western blotting assay.

FIG 8. hIL-17RLM-L interferes with Ras-MAPK signalling by acting on the upstream molecules of Ras. Constituted activation was detected by immunoblotting. The whole lysates of transfected cells with active MEK (B) or active Ras (D) were immunoblotted with anti-p-ERK, anti-ERK and anti-IL-17RLM rabbit polyclonal serum, respectively. Constitutively active MEK (MEK1RF)(A, B) or constitutively active Ras (Ras G12V)(C, D) constructs were transiently co-transfected into PC12 cells with Elk-1 luciferase reporter plasmids or the increasing amounts of hIL-17RLM-L plasmid for 36 hrs. The luciferase activity was measured as described above. Additionally, ERK

FIG 9. Schematic representation of the signaling potential and biological events mediated by IL-17RLM. EPOR/IL-17RLM chimeric construct leads to STAT5b activation mediated by Jak's response to erythropoietin(EPO). The artificial homodimerization of the IL-17RLM cytoplasmic domain is sufficient for growth of Ba/F3 cells. Additionally, overexpression of full length IL-17RLM or the cytoplasmic domain could inhibit the FGF-induced PC12 cell differentiation possibly through the prevention of FGFR-Ras-MAPK signaling pathway. Our data show IL-17RLM interferes with Ras-MAPK signaling pathway by interacting with FGFR or acting on

the up-stream signaling molecules of Ras.

DETAILED DESCRIPTION OF THE INVENTION

In accordance with an aspect of the present invention, there is provided a novel polypeptide, as well as biologically active and diagnostically or therapeutically useful fragments thereof. The polypeptide of the invention is of human origin and designated as hIL-17RLM. hIL-17RLM has two alternative splicing forms, namely hIL-17RLM-L and hIL-17RLM-S, with an amino acid sequence of SEQ ID NO:2 and SEQ ID NO:4, respectively. hIL-17RLM-L shares a 75% amino acid sequence identity with hIL-17RLM-S.

The term "fragment" when referring to the polypeptides of SEQ ID NO:2 or SEQ ID NO:4, means a polypeptide which retains essentially the same biological function or activity as such polypeptides.

The polypeptide of the present invention may be a recombinant polypeptide, a natural polypeptide or a synthetic polypeptide, preferably a recombinant polypeptide.

The polypeptides of the present invention include the polypeptide of SEQ ID NO:2, the polypeptide of SEQ ID NO:4 as well as polypeptides which have at least 70% similarity (preferably at least 70% identity), and more preferably at least 90% similarity (more preferably at least 90% identity) and still more preferably at least 95% similarity (still more preferably at least 95% identity) to the polypeptide of SEQ ID NO:2 or SEQ ID NO:4. The terms "similarity" and "identity" are used herein with the meanings known in the art.

Computer-assisted analysis suggests that mature IL-17RLM-L contains a putative signal peptide of 16 amino acids, a 281-amino acid extracellular domain (C17-Pro297), a 23-amino acid transmembrane stretch (Ile298-Met320), and a 420-amino acid longer cytoplasmic tail (Cys321-Leu739) than that of IL-17BR/IL-17Rh1. The cytoplasmic portion of this new receptor polypeptide of the invention is much longer than IL-17BR, and is comparable with the unusually long tail described for IL-17 receptor. Additionally, there are nine cystine residues in extracellular domain and eight potential N-linked glycosylation sites in the extracellular domain of the polypeptide of the invention. The extracellular domain also consists of a predicted immunoglobulin domain and a putative fibronectin III domain. This protein is predicted to be a type I membrane protein according to Hartmann membrane topology model and PSORT II server prediction. But there is no WSXWS motif, typical of type I receptor (32,33) in the extracellular domain. The sequence of IL-17RLM-L is slightly atypical for type I cytokine receptors in that the usual WSXWS motif is replaced by WSPGA. Furthermore, a segment (TPPPLRPRKVW) located proximal to the IL-17 receptor transmembrane domain, which is highly conserved among cytokine receptor, is replaced by the proline-rich

motif (PFHPPPLRYREP), which was a typical feature of a transactivation domain for transcription factors. Interestingly, both a putative TIR domain (Toll/IL-1-Receptor homology domain) and a putative SH3 interaction domain (proline-rich domain) were predicted in the intracellular domain of the protein from (V358 to K424). Additionally, a putative tyrosine phosphorylation site juxtaposed to the transmembrane domain (Y329) was also identified. The long COOH-terminal tail (C-tail) of IL-17RLM also contains multiple tyrosine residues and putative Stat binding motifs.

In accordance with another aspect of the present invention, there is provided an isolated nucleic acid which encodes the polypeptide having the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4.

The polynucleotide of the invention was discovered in normal human testes mRNA and 293T cells mRNA by 5'-RACE assay. It belongs to human IL-17 receptor family and has a higher similarity to IL-17R than that of all known IL-17 receptor homologues. At least two RNA splicing variants were found, transcribed from 13 exons localized on human chromosome 3p21.1, named by hIL-17RLM-L and hIL-17RLM-S, respectively. The longer isoform of IL-17RLM (IL-17RLM-L) contains an open reading frame encoding a polypeptide of 739 amino acid residues. The shorter isoform of IL-17RLM (IL-17RLM-S) contains an open reading frame encoding a polypeptide of 595 amino acid residues.

The polynucleotide of the present invention may be in the form of RNA or in the form of DNA, which DNA includes cDNA, genomic DNA and synthetic DNA. The DNA may be double-stranded or single-stranded, and if single stranded may be the coding strand or the non-coding strand. The coding sequence which encodes the longer isoform IL-17RLM-L may be identical to the coding sequence shown in SEQ ID NO:1 or may be a different coding sequence which coding sequence, as a result of the redundancy or degeneracy of the genetic code, encodes the same polypeptide as the DNA of SEQ ID NO:1. The coding sequence which encodes the shorter isoform IL-17RLM-S may be identical to the coding sequence shown in SEQ ID NO:3 or may be a different coding sequence which coding sequence, as a result of the redundancy or degeneracy of the genetic code, encodes the same polypeptide as the DNA of SEQ ID NO:3.

The present invention further relates to polynucleotides which hybridize to the hereinabove-mentioned sequences if there is at least 70%, preferably at least 90%, and more preferably at least 95% identity between the sequences. The present invention particularly relates to polynucleotides which hybridize under stringent conditions to the hereinabove-mentioned polynucleotides. As herein used, the term "stringent conditions" means hybridization will occur only if there is at least 95% and preferably at least 97% identity between the sequences. The polynucleotides which hybridize to the above-mentioned polynucleotides in a preferred embodiment encode polypeptides

which either retain substantially the same biological function or activity as the polypeptides encoded by the DNAs of SEQ ID NO:1 or SEQ ID NO:3.

The present invention also relates to vectors which include polynucleotides of the present invention, host cells which are transfected or transformed with vectors of the invention and the production of polypeptides of the invention by routine recombinant techniques.

The present invention also relates to diagnostic or therapeutic uses of the polypeptide of the invention. As shown in the Examples, the polypeptide of the invention is highly expressed in kidney or testis related carcinoma tissues. Therefore, the polypeptide or its encoding polynucleotide may be employed as a diagnostic marker in detecting these cancers. Furthermore, as shown in FIG. 6A, stably expressed hIL-17RLM-L significantly inhibits FGF2 or NGF-induced rat pheochromocytoma PC12 cell differentiation, so the polypeptide of the invention may be useful in treating neuron diseases. Additionally, being similar to IL-17 receptor which is known to be involved in autoimmune diseases such as rheumatoid arthritis and asthma, the polypeptide of the invention may be antagonized so as to treat these diseases. Preferably, the antagonist is an antibody. Alternatively, antisense technique may be employed to prevent the *in vivo* expression of the polypeptide of the invention so as to treat these autoimmune diseases.

The present invention will be further described with reference to the following examples; however, it is to be understood that the present invention is not limited to such examples.

Examples

In the examples, the following experimental procedures are used to clone and identify the polynucleotide of the invention and to test the functions of the polypeptide of the invention:

Genebank database search and RACE PCR The human high-throughput genomic sequence was scanned for "virtual" ORFs by using ab initio gene prediction program GENSCAN (30). The signal sequence was predicted by SIGNALP algorithm(31). Transmembrane predictions were performed with TMPRED (32), an algorithm based on the statistical analysis of a transmembrane domain database pT-A e, as well as TMHMM algorithm. Netphos2.0 was used to identify and score all possible cytoplasmic serine, threonine and tyrosine phosphorylation sites. To obtain a full length of cDNA, 5'-RACE PCR was performed using mRNA from normal human testis and 293T cells according to SMARTTM RACE cDNA amplification kit user manual (CLONTECH). Total RNA was extracted by using TRIzol reagent kit (Life Technologies, Inc) and reverse transcribed by using an oligo (dT) primer and superscript II (Life Technologies, Inc). The 5'-RACE PCR products were then cloned

into dv vector according to AdvanTageTMPCR cloning kit instructions and then sequenced.

Cell lines THP-1 is a human acute monocytic leukemia cells. Jurkat and 6T-CEM are T cell acute lymphocytic leukemia cell lines. K562 is an erythroleukemia cell line. U937 is human leukemia cell line. 786-O is a renal cell adenocarcinoma cell line. GRC-1 is a granular renal carcinoma cell line. THP-1, Jurkat, 6T-CEM, K562, U937 and 786-O cells were maintained in RPMI 1640 supplemented with 10% FBS, 2mM L-glutamine/penicillin/streptomycin (Life Technologies, Inc) at 37°C under 5% CO₂. GRC-1, HepG2, A431, 293T cells and COS7 cells were maintained in DMEM containing 10% FBS/glutamine/penicillin/streptomycin. Ba/F3 is a murine IL-3-dependent pro-B cell line, and grown in RPMI complete medium containing 5×10⁻⁵ M 2-mercaptoethanol and 5% WEHI-3B-conditioned medium as a source of IL-3. Rat pheochromocytoma PC12 cells (ATCC: CRL-1721) were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum and 5% horse serum (Life Technologies, Inc).

Antibody preparation For bacterial production of hIL-17RLM, an open reading frame coding for the peptide in intracellular domain of hIL-17RLM (M³²⁰-I⁴⁵⁷, 138aa) was amplified by PCR and cloned into pGEX4T-1 vector. The reading frame was sequence-confirmed following cloning. hIL-17RLM was expressed in the inclusion bodies in *E.coli* and solubilized with 4 M guanidine HCl and dialyzed against 50 mM sodium acetate buffer, pH5, containing 0.1 M NaCl. Antisera were raised in rabbits by standard methods and used for immunoblot analysis after 1000 to 10000-fold dilution.

Immunofluorescent staining and microscopy 786-O and GRC-1 cells were cultured on 6-well plate with 8×10⁴ per well (Corning Incorporated Corning, NY). One day later, cells were washed in PBS and fixed with 4% formaldehyde in PBS for 20 minutes. Then, the cells were permeabilized with 0.5% Triton X-100 in PBS for 10 minutes. Cells were blocked with 10% normal goat serum in PBS for 1 hour and then incubated with 1:1000 diluted anti-IL-17RLM antibody for 1 hour. After washed with 0.1% Tween-20 in PBS, cells were incubated with 1:100 diluted FITC-labeled goat anti-rabbit Ig G (Santa Cruz Biotechnology, Inc) in PBS. After incubated for 1 hour, cells were washed with 0.1% Tween-20 in PBS again. The cells were viewed using Nikon inverted microscope ECLIPSE TE300.

Generation of stably transfected Ba/F3 and PC12 cell clones

The murine EPOR cDNA was obtained from Harvey Lodish's lab, and cloned into pIREneo expression plasmid by PCR amplification with primers as follows: sense, 5' TATAGCGATATCATGGACAAACTCAGGGTGCC 3' (SEQ ID NO:5), antisense, 5'AATGAATTCC TAGGAGCAGGCCACATAGCC 3' (SEQ ID NO:6). Full length cDNA of human IL-17RLM-L was cloned into pcDNA3 expression plasmid with primers as follows: sense, 5' ATAAAGCTTATGGCCCCGTGGCTGC 3' (SEQ ID

NO:7), antisense, 5' TTCTCGAGTTACAAAGGGGCGACCGC 3' (SEQ ID NO:8). Chimeric construct of EPOR/IL-17RLM containing the extracellular and transmembrane domain of EPOR and intracellular domain of hIL-17RLM was prepared by PCR amplification with specific primers as follows: for EPOR (extracellular domain), sense, 5' TATAGCGATATCATGGACAAACTCAGGGTGCC 3' (SEQ ID NO:9), antisense, 5' TATAGAATTCCAGCAGGGCCAGAACCGTC 3' (SEQ ID NO:10), for hIL-17RLM (intracellular domain), sense, 5' ATTGAATTCTGCCGCAAGAAC 3' (SEQ ID NO:11), antisense, 5' ATTGGATCCTTACAAAGGGGCGACCGC 3' (SEQ ID NO:12). Above constructs (20 μ g) were stably introduced into 10⁷ Ba/F3 cells by electroporation using a BTX machine at 240 V and 1050 μ F. Twenty-four hours after electroporation, cells were re-plated in growth medium supplemented with 1mg/mL of G418. G418 resistant cells were kept in medium with IL-3 and G418 or switched to a given cytokine at the indicated concentration: human IL-3, 5ng/mL, rhEPO, 2 U/mL. The selected positive clones were confirmed by Northern blot and immunoblotting.

To establish hIL-17RLM-L or EPOR/IL-17RLM stably expressed PC12 cell lines, PC12 cells were transfected with above constructs using with Transfast transfection reagent (Promega). Forty-eight hours post-transfection, cells were plated at several different dilutions in media containing 0.5 mg/ml G418. For the next two weeks, the selective media were replaced every 3 to 4 days. Once the distinct "islands" of surviving cells were visualized, the individual clones were transferred into 96 well plates and continued to maintain cultures in selected media. The positive clones were confirmed by immunoblotting.

Northern blot analysis and reverse transcription-PCR analysis Multiple tissue blots containing poly (A)⁺RNA (2 μ g per lane) from various human tissues were purchased from CLONTECH. A fragment of IL-17RLM coding regions was used as hybridization probe. DNA probes were labeled with [α -³²P] dCTP according to Prime-a-Gene[®] labeling system instructions(Promega). Labeled nucleotides were purified using with MicroSpinTMG-50 columns (Amersham Pharmacia Biotech). Hybridization was performed with Expresshyb (CLONTECH) at 68°C for 1 hr. The blots then were washed with 2 \times SSC/0.05% SDS solution at room temperature for 40 min, followed by washing in 0.1 \times SSC/0.1% SDS solution at 50°C for 40 min with one change of fresh solution. The blots were exposed in Phosphor Imager for 24 hrs. DNA probes from blots were stripped by incubating the blot in sterile H₂O containing 0.5% SDS at 100°C for 20 min. Then the membrane was additionally blotted by β -actin probe as internal control.

Total RNA from all selected cell lines was extracted using with TRIzol reagent, according to the manufacturer's recommendations (Life Technologies). RT-PCR was performed on 0.5 μ g of total RNA with an oligo (dT) primer according to QIAGEN[®]OneStep RT-PCR kit handbook instructions. cDNA was amplified for 35 cycles with primers specific for the CDS of human IL-17RLM as follows: sense 5'-ATAGGTACCATGGAATCTAACCTTCCTG-3' (SEQ ID NO:13) and antisense 5'-ATAGGATCCAAAGGGGCGACCGCG-3' (SEQ ID NO:14). Then the nested PCR reactions were performed using previous PCR products as a template with

primers specific for a 500bp coding region of human IL-17RLM cDNA as follows: sense 5'-CGTGGTACCGATGGAATCTAACCTTCCTG-3' (SEQ ID NO:15) and antisense 5'-ATATCTAGAGGGCCGGCCCACGG-3' (SEQ ID NO:16). The upstream primer of human β -actin is 5'-AGCTCACCATGGATGATGATATC-3' (SEQ ID NO:17), and its downstream primer is 5'-TGTTGAAGGTCTAAACATGATCT-3' (SEQ ID NO:18), with the expected product of 394 bp. The PCR products were subjected to electrophoresis on 1.8% ethidium bromide-stained agarose gel, then transferred to nylonmembrane, and probed with a 32 P-labeled cDNA probe specific for IL-17RLM.

Preparation of nuclear and non-nuclear protein fractions EPO- stimulated cells were washed and scraped into phosphate-buffered solution and centrifuged at 4,500 rpm for 5 min in a microfuge. The cells were suspended in buffer (10mM Tris, pH 7.5, 1.5 mM MgCl₂, 10mM KCl, 0.5% Nonidet P-40) at about 10× the packed cell volume and lysed by gentle pipetting. Nuclei were recovered by microcentrifugation at 7,000 rpm for 5 min. The supernatant, which represents the cytoplasmic and membrane protein fraction, was collected and stored at -80°C. Nuclear proteins were extracted at 4°C by gentle suspension of the nuclei (at about 2× the packed nuclear volume) in buffer containing 20 mM Tris (pH 7.5), 10% glycerol, 1.5 mM MgCl₂, and 420mM NaCl, 0.2 mM EDTA, followed by 30 min of platform rotation. The nuclear protein suspension was cleared by centrifugation at 13,000 rpm for 15 min. The supernatants were collected and frozen at -80°C or directly used in gel shift assays. All buffers contained the following additions: 1-2 μ g/ml each of aprotinin, leupeptin, pepstatin, 0.2 mM PMSF, 0.5mM DTT, 10mM NaF and 0.1 mM Na-vanadate. All steps were carried out on ice or at 4°C. Protein amounts were kept in the same for different treatments after measured by BCA protein assay (PIERCE), using BSA as a standard.

Electrophoretic mobility-shift assay DNA probes [double-stranded β -casein promoter GAS (γ -interferon activated sequence): 5'-AGATTTCTAGGAATTC-3'] were prepared by end-labeling with [γ - 32 P]ATP and T4 polynucleotide kinase and purified by G-50 MicroSpin columns. Cells were washed three times in PBS and starved in the absence of cytokines for 8 hrs in RPMI 1640. Cells were then stimulated for the indicated times with the indicated cytokine at a concentration used in growth medium. Typically, 5 μ l (10-20 μ g) of nuclear proteins was incubated with 100,000 cpm of 32 P-labeled oligonucleotides for 2 hrs at room temperature. The nuclear proteins and various oligonucleotide probes were incubated in buffer containing 10 mM Tris (pH 7.5), 10% glycerol, and 0.2% Nonidet P-40. Additionally, 2-4 μ g of poly (dI-dC) was included as a nonspecific competitor DNA. Protein-DNA complexes were resolved on 4% nondenaturing polyacrylamide gels in 0.5×TBE running buffer. After electrophoresis, gels were dried and subjected to autoradiography. Antibody supershift experiments were carried out by addition of 4 μ l of various antibodies purchased from Santa Cruz Biotechnology.

Western blotting and immunoprecipitation The cells were lysed in lysis buffer containing 50mM Tris, pH 7.6, 150 mM NaCl, 1% NP-40 and 1mM sodium orthovanadate in the presence of protease inhibitors. The immune complexes were captured with protein A or G Sepharose, washed in lysis buffer and resolved by

SDS-PAGE. The proteins were transferred onto nitrocellulose membrane and the membrane was blocked with 5% non-fat milk in PBS-buffered saline containing 0.1% Tween-20 overnight at 4°C and then incubated the indicated primary antibody followed by horseradish-peroxidase-conjugated rabbit anti-sheep or anti mouse antibodies as secondary antibodies and detected by chemoluminescence according to manufacturer's instructions (ECL; Amersham).

Luciferase assay 4FTKSLN-Luc is a STAT5- responsive luciferase reporter plasmid. This construct contains 4 copies of IGAS sequence inserted upstream of a luciferase gene controlled by the thymidine kinase (TK) promoter. As an internal control, we used the pRL-TK vector (Promega) containing the *Renilla* luciferase gene under the control of the TK promoter. Cos7 cells were seeded into 12-well plates at a density of 5×10^4 cells/well, and transfected by SuperFect®Transfection reagent (Qiagen) with the indicated constructs. The amounts of transfected DNA were kept constant in all transfections. After 36 hrs of transfection, the cells were stimulated with or without 2 U/ml of recombinant human EPO (R&D Systems, Inc) for 30 min. Cells were collected and lysed for measurement of arbitrary luciferase activities. Luciferase activity was monitored with the Dual-Luciferase Reporter assay System kit (Promega). Each experiment was repeated three times. Data were normalized by co-transfection with *Renilla* luciferase reporter vector and expressed as mean \pm S.D.(n=3).

The Elk-1 luciferase activity assay was performed using trans-reporting constructs including PFA-Elk-1 and PFR-luciferase plasmids (PathDetect *in vivo* signal transduction pathway trans-reporting system, Stratagene) according to the manufacture instructions. The Elk-1 luciferase activity was measured using a Luciferase Assay System (Promega). The results were expressed as mean \pm S.D. from three independent experiments.

Cell proliferation assay DNA replication was analyzed by incorporation of [methyl^3H] thymidine (Amersham Pharmacia Biotech). Typically, Ba/F3, Ba/F3/EPOR and BA/F3/EPOR/IL-17RLM cells were washed three times in PBS, then plated into 96 well-plate (2×10^4 cells/200 μl /well) and starved for 8 hrs in RPMI 1640 medium containing 5% FCS, treated with the indicated concentration EPO for 72 hrs. [Methyl^3H] thymidine was added into the medium for 4 hrs. [^3H] thymidine incorporation was measured by Microplate Scintillation & Luminescence Counter (Packard). For time course and dose dependence cell proliferation experiments, 5,000 cells/200 μl /well were stimulated with or without 0.5 U/ml of EPO for various time. Experiments were performed in triplicate.

Differentiation of PC12 cells PC12 cells were maintained in DMEM supplemented with 10% fetal calf serum, 5% horse serum, and 4.5 g/L glucose at 37°C under 5% CO₂. The cells were plated at a sub-confluent density on 12-well culture plates coated with poly-L-lysine to improve cell attachment activity. The next day, cells were transiently transfected with EGFP, wild type IL-17RLM-L and the mutants for 36 hrs using Effectene™ Transfection reagent (Qiagen). Cells were stimulated with or without 20ng/ml of recombinant human FGF2 (R&D) for 72 hrs, then examined by fluorescence microscope. Cells with processes longer than 1.5 times the diameter of the cell body were considered to be positive for neurite outgrowth. The numbers of

undifferentiated and differentiated cells were counted in three randomly selected fields, containing approximately 200 cells each. Data were expressed as means \pm S.D of three independent counts.

Immunohistochemistry Various normal human tissues were from the surgery of different patients in The First People's Hospital, Peking Medical University. All tissue specimens were formalin-fixed and paraffin-embedded. Rabbit polyclonal antibody (anti-intracellular domain of hIL-17RLM) generated was used. DAKO EnVisionTM, well suited for a two step immunohistochemistry procedure, was used for immunohistochemistry assay according to the kit instructions. The color development (varying from 10-30 min) was stopped under microscopic examination by adding a mixture of 10mM Tris-HCl (pH 8.0) and 1mM EDTA. The slides were mounted immediately. Pre-immune serum was used as control for each sample.

Example 1 Cloning and primary structure of hIL-17RLM

To explore the existence of additional members of the IL-17 receptor family, we screened the EST database and the NR database in NCBI Gene Bank using the cytoplasmic domain of IL-17 receptor by tBlastn and Blastp algorithm. We found several ESTs encoding an unknown protein reported in the Gene Bank as hypothetical human protein DKFZp434N1928 (AL133097). However this sequence was only a fragment without N-terminus. Then we performed 5'-RACE PCR assay using the mRNA from the human testis tissue and 293T cells, and obtained two complete cDNAs with 4477 bp and 4478 bp in length. The full length ORF of 4477 bp sequence encoded a novel single transmembrane protein of 739 amino acids (FIG 1A), while the other cDNA encoded a protein of 595 amino acids, which was a truncated form, lacking 144 amino acids at the N-terminus compared with the 739-amino acid protein (FIG 1B). BLAST analysis revealed that the most related protein with the two novel proteins was IL-17 receptor (IL-17AR). So the two proteins were named as human IL-17 receptor like molecule long form (hIL-17RLM-L) and short form (hIL-17RLM-S), respectively. The pair-wise comparison of the protein sequences of hIL-17RLM-L and hIL-17AR by BLASTP algorithm using BLOSUM62 scoring matrix showed that hIL-17RLM-L shared 31% identities (47% similarities) to hIL-17AR (FIG 1C). Additionally, hIL-17RLM was mapped on human chromosome 3p21.1. Genomic structure analysis showed that hIL-17RLM-L was comprised of 13 exons and spanned 70903 base pairs, while hIL-17RLM-S consisted of 14 exons and spanned 85485 base pairs (FIG 1D). Both of the sequences were submitted to Gene Bank with the accession number of AF494208 and AF494211, respectively.

We also cloned the mouse orthologues of the gene named as mIL-17RLM-L and mIL-17RLM-S with 738 and 594 amino acids, respectively. The Gene Bank accession numbers are AF494210 and AF494209. hIL-17RLM-L shared 75% of identities to mIL-17RLM-L at amino acids level. mIL-17RLM-L was located on chromosome 14 with 13 exons and spanned 66304 base pairs. However mIL-17RLM-L was identical to the newly defined mSef gene, which shared 46% of identities with zSef gene(27, 28).

Computer-assisted analysis suggested that hIL-17RLM-L contained a putative signal peptide of 16 amino acids, a 281-amino acid extracellular domain (C¹⁷-Pro²⁹⁷), a 23-amino acid transmembrane stretch (Ile²⁹⁸-Met³²⁰), and a 420-amino acid cytoplasmic tail (Cys³²¹-Leu⁷³⁹). The cytoplasmic portion of this new receptor was much longer than that of IL-17BR, and comparable with the unusually long tail of IL-17AR. This protein was predicted to be a type I cytokine receptor according to Hartmann membrane topology model and PSORT II server. However, hIL-17RLM-L had a WSPGA instead of WSXWS motif, which is a typical motif in the extracellular domain of type I cytokine receptors (33, 34). There were eight cystine residues and nine potential N-linked glycosylation sites in the extracellular domain, where an immunoglobulin domain and a fibronectin III domain were also predicted. Furthermore, a highly cytokine receptor conserved segment (TPPPLRPRKVV) located proximal to the IL-17 receptor transmembrane domain was replaced by the proline-rich segment (PFHPPPLRYREP), a putative SH3 interaction domain, which was a typical feature of a transactivation domain for transcription factors. Additionally, a putative TIR domain (V³⁵⁸ to K⁴²⁴) (Toll/IL-1Receptor domain) and a putative TRAF6 binding motif (P³⁴⁷ to L³⁵¹), Pro-X-Glu-X-X (aromatic/acidic residue) were predicted in the intracellular portion of hIL-17RLM-L. The TRAF6 binding motif was found in TRANCE-R and IRAK adapter kinases for ILR/Toll-like receptor signaling(35), suggesting that hIL-17RLM may play a role in the Toll-like receptor signaling. The long COOH-terminal tail (C-tail) of hIL-17RLM also contained multiple tyrosine residues. All of these implied that the protein might be a novel signaling receptor.

Example 2 Cellular and tissue distribution of the hIL-17RLM

To gain insight into the potential function of this receptor, we examined the tissue distribution of this receptor by Northern blot using an open reading frame-specific probe for hIL-17RLM. As shown in FIG 2A, the mRNA was abundant with two specific transcripts of ~8.5kb and ~4.5kb in testis and kidney tissues. However, transcripts were detected at lower levels in brain, spleen, heart and uterus, and barely detected in lung, thymus and peripheral blood lymphocytes.

To observe the IL-17RLM mRNA levels in different cells, we carried out RT-PCR analysis. As shown in FIG 2B, mRNA of IL-17RLM was observed in most tumor and normal cell lines, including Jurkat, A431, GBE, 6T-CEM, K562, Hela, Ho8910, SGC7901, CNE, HepG2, 293, L02, CHO, COS1, but not HL60, THP-1, and U937 cell lines. However, IL-17RLM mRNA was hardly detected in those cell lines by Northern blot (data not shown), suggesting that IL-17RLM was weakly expressed in these cell lines.

The predicted molecular masses of hIL-17RLM-L and hIL-17RLM-S were 82 kD and 67kD, respectively. A single species of approximately 85kD or 70kD was observed

respectively when hIL-17RLM-L or hIL-17RLM-S construct was translated *in vitro*. (FIG 2C, left). In order to monitor hIL-17RLM expression *in vivo*, a rabbit polyclonal antiserum against a fragment of intracellular domain of hIL-17RLM was generated and used for Western blot. The data showed that the protein was approximately 100 kD, which was larger than the *in vitro* translated products. This was consistent with many N-linked glycosylation sites in the extracellular domain of hIL-17RLM-L (FIG 2C, right).

To examine the subcellular location of hIL-17RLM proteins, we carried out immunostaining assay in a renal cell adenocarcinoma cell line 786-O and a granular renal carcinoma cell line GRC-1 using the rabbit polyclonal antiserum. The data showed that the novel protein was mainly localized in the cell membrane (FIG 2D).

To investigate the hIL-17RLM protein expression in different human normal tissues, we carried out immunohistochemical assay using the rabbit polyclonal antiserum. The data showed that hIL-17RLM protein was mainly expressed in kidney, testis and liver, but not detectable in breast, skin and endometrium (Figure 2E). Interestingly, hIL-17RLM protein was specifically expressed in some types of cells in the process of spermatogenesis. IL-17RLM protein was detected in spermatogonia, spermatocytes and spermatids in testis. However, it was not detectable in spindle-shaped myofibroblasts and fibroblasts in the basement membrane of the seminiferous tubule. Also, it was negative result in Leydig cells in the supporting tissue of testis. In the blood vessels of testis, hIL-17RLM was not expressed in the smooth muscle cells but weakly detected in endotheliocytes. In kidney, hIL-17RLM protein was mainly expressed in the proximal convoluted tubule. It was not detected in the capillaries but weakly expressed in the mesangium in the renal corpuscle. In liver, it was clear that hIL-17RLM was expressed uniquely in hepatocytes while there was no expression in other cells such as the simple cuboidal or columnar epithelium.

Example 3 Signaling potential of STAT 5 activation by IL-17RLM

It is noteworthy that IL-17RLM contains a SH3 interaction domain and many potential tyrosine phosphorylated sites in the intracellular domain of this protein. We reasoned that IL-17RLM might signal the STAT pathway. To test this hypothesis, we constructed a chimeric molecule fusing the extracellular and transmembrane domains of erythropoietin receptor with the intracellular domain of IL-17RLM. We carried out luciferase assay using a STAT5-responsive luciferase reporter plasmid transfected into COS7 cells. As shown in FIG 3A, this chimeric receptor (EPOR/hIL-17RLM) mediated STAT5 activation significantly upon EPO stimulation. Moreover, STAT5 CYF, a dominant mutant of STAT5, could inhibit STAT5 activation, suggesting that the chimeric receptor specifically activated STAT5.

Next we evaluated whether the chimeric molecule (EPOR/hIL-17RLM) could mediate STAT5 tyrosine phosphorylation in response to EPO. STAT5 was

immunoprecipitated with anti-STAT5 antibody and blotted with anti-phosphotyrosine (anti-PY). The results showed that both the EPOR and EPOR/hIL-17RLM could mediate STAT5 tyrosine phosphorylation in the presence of EPO (FIG 3B, up panel) while the equal amounts of STAT5 or JAK protein were expressed (FIG 3B, middle panels), suggesting that IL-17RLM may signal through initiation of STAT5 tyrosine phosphorylation.

We further performed electrophoretic mobility-shift assay to investigate whether EPOR/hIL-17RLM chimeric receptor could increase STAT5 DNA-binding activity upon the stimulation of EPO. As shown in FIG 3C, both EPOR and EPOR/hIL-17RLM stimulated STAT5 binding strongly to DNA in the presence of EPO (FIG 3C, lanes 4 and 6). The specificity was confirmed by adding cold probes as competitors (FIG 3C, lanes 7 and 8) or by adding anti-STAT5 antibody for supershift (FIG 3C, lanes 9 and 10). As is known that STAT5 might form homo-dimer or heter-dimer with other STATs, we tested the shift band by adding anti-STAT1 (FIG 3C, lane 11) or anti-STAT3 (FIG 3C, lane 12) antibody. The results showed that none of the anti-STAT1 or anti-STAT3 antibody could drive the band to supershift, suggesting that EPOR/hIL-17RLM stimulated STAT5 homodimerization. The specificity for the STAT5 activation was further confirmed by the overexpression of STAT5 CYF, which blocked STAT5 activation (FIG 3C, lanes 15 and 16).

To detect the signal potential of the endogenous STAT5 activation mediated by EPOR/hIL-17RLM chimeric receptor, the EPOR/hIL-17RLM or EPOR was stably transfected into Ba/F3 cells and the gel shift assays were performed. The results showed that STAT5 was activated in a time course in the presence of EPO (FIG 3D). Importantly, when we detected the activation of endogenous STAT5, we found that EPOR/hIL-17RLM was more likely to stimulate STAT5b rather than STAT5a (FIG 3E, lanes 9, 10 and 11). Altogether, these data suggested that the novel receptor could mediate STAT5 signal pathway.

Example 4 Chimeric hIL-17RLM promotes cell proliferation in BA/F3 cells

To address whether IL-17RLM was capable of transmitting a proliferation signal, we set up Ba/F3/EPOR and Ba/F3/EPOR/hIL-17RLM stably expressed cell lines. We carried out the cell proliferation assay using those cells. As shown in Fig 4A, the Ba/F3 cells had no proliferative response to EPO, while the Ba/F3/EPOR cells had strong proliferative response. Interestingly, the Ba/F3/EPOR/hIL-17RLM cells had also a significant proliferative response to EPO in a dose dependent manner (FIG 4A) and a time course of manner (FIG 4B). Moreover, the Ba/F3/EPOR/hIL-17RLM cells could be maintained for a longer time in presence of low dose of EPO (data not shown). These results suggested that IL-17RLM could be a novel signaling receptor that could signal Ba/F3 cell proliferation. Based on the result that EPOR/hIL-17RLM specifically activated STAT5, we reasoned that EPOR/hIL-17RLM stimulated Ba/F3

cell proliferation possibly through the activation of STAT5 pathway.

Example 5 IL-17RLM interacted with FGFR

It has been reported that FGFRs were highly expressed in kidney tissue (36), where hIL-17RLM was abundant also(FIG 2A and E). Based on the fact that mSef interacted with FGFRs (28), we reasoned that hIL-17RLM could also interact with FGFRs and affect FGF signaling. To detect whether the physical interaction of the two receptors occurred, we carried out co-immunoprecipitation assay by co-expression of the two proteins in Cos7 cells. We successfully precipitated the FGFR1 (FIG 5A) and FGFR2 (FIG 5B) using anti-IL-17RLM serum but failed to precipitate FGFR3 (data not shown), suggesting that IL-17RLM specifically interacted with FGFR1 and FGFR2 in the intact cells. These results were consistent with the report about the interaction of zebrafish zSef with xenopus FGFR1 or FGFR2 (28). It also implied that hIL-17RLM might elicit a similar effect on FGFR signaling to Spred or Sprouty family members, which inhibited FGFR signaling strongly (37-40).

To furthermore examine whether the co-expression of the two receptors occurred in human tissue, we carried out the immunostaining assay with the anti-FGFR1 antibody and anti-hIL-17RLM serum. The results demonstrated that hIL-17RLM was co-expressed with FGFR1 in kidney (FIG 5C), suggesting that hIL-17RLM co-localized with FGFR1 in normal human tissue. Interestingly, when we over-expressed both hIL-17RLM and FGFR2, we found the complete co-localization of the two proteins (FIG. 5D). Taken together, these data suggested that hIL-17RLM interacted with FGFR1 and FGFR2 but not FGFR3.

Example 6 hIL-17RLM-L strongly inhibits basic FGF2 or NGF-induced PC12 cell differentiation.

Based on the observation that hIL-17RLM was able to interact with FGFR1 and FGFR2, we examined whether hIL-17RLM could affect FGF signaling. We stably expressed hIL-17RLM-L in PC12 cells, a rat pheochromocytoma cell line which could be induced into sympathetic neuron-like cells possessing elongated neuritis by basic fibroblast growth factor (FGF-2) or NGF. The data showed that hIL-17RLM elicited strong inhibitory effects on the differentiation of PC12 cells (FIG. 6A). When the differentiated cell numbers were calculated, it was obvious that the inhibitory effects of hIL-17RLM on the differentiation could be sustained even when the cells were exposed to higher dose of FGF2 or NGF (FIG. 6A, and 6B). When the cells were exposed to FGF2 or NGF for a longer time, it was still significant that hIL-17RLM inhibited the PC12 cell differentiation (FIG. 6C and 6D). All the results indicated hIL-17RLM could significantly inhibit the differentiation of PC12 cells stimulated by

FGF2 or NGF, even in the condition of higher dose or prolonged exposure.

To further determine which domain of hIL-17RLM was necessary for the inhibition of PC12 cell differentiation induced by FGF2, we constructed the N-terminal truncated mutant hIL-17RLM-L(ΔN), which lacks N-terminal extracellular domain of hIL-17RLM, the C-terminal truncated mutant hIL-17RLM-L(ΔC), which lacks C-terminal intracellular domain of hIL-17RLM, and a mutant hIL-17RLM-L(DN), which lacks a motif (E327~L333) in the intracellular domain containing a putative tyrosine phosphorylation site. We overexpressed these mutants with EGFP in PC12 cells in the presence or absence of FGF. The results showed that overexpression of hIL-17RLM-L(ΔN) or hIL-17RLM-L(DN) had similar inhibitory effects on FGF2-induced PC12 cell differentiation, as in the case of hIL-17RLM-L(WT) (FIG. 6E and 6F). However, overexpression of hIL-17RLM-L(ΔC) had no inhibitory effects on the differentiation of PC12 cells, suggesting that the intracellular domain played a critical role in the inhibition of PC12 cell differentiation induced by FGF2. The results also suggested hIL-17RLM-L(DN), which lacks a motif (E327~L333) in the intracellular domain of hIL-17RLM-L, may not function as a dominant negative form.

Example 7 hIL-17RLM inhibited Ras-MAPK signaling pathway.

It has been reported Ras-MAPK signaling was required for FGF2-induced PC12 cell differentiation. To investigate the role of hIL-17RLM in MAPK activation during FGF2-induced PC12 cell differentiation, we detected the effects of IL-17RLM on Elk-1 mediated luciferase activity. The data showed that overexpression of hIL-17RLM significantly suppressed FGF2-induced Elk-1 luciferase activity in PC12 cells (FIG. 7A), which is comparable to the role of Sprouty 4 (37-39). Compared with hIL-17RLM-L, the C-terminal truncated mutant hIL-17RLM-L(ΔC) and the N-terminal truncated mutant hIL-17RLM-L(ΔN) had about 23% and 81% of the inhibitory effect, respectively (FIG. 7A column 4,5). The result was also correlated with the inhibitory effect of hIL-17RLM on the FGF2-induced PC12 cell differentiation. Furthermore, our results showed both hIL-17RLM-L(WT) and hIL-17RLM-L(ΔN) suppressed FGF2-dependent Elk-1 luciferase activity in PC 12 cells in a dose-dependent manner (FIG. 7B and 7C).

Earlier studies showed that activation of ERK1/2 was important for neurite outgrowth in PC12 cells, and ERK1/2 phosphorylation was strongly but transiently induced by FGF2, with the level of phosphorylation reaching a maximum within 5-10 minutes and then declining to lower sustained levels. With this in mind, we examined whether hIL-17RLM-L suppressed endogenous ERK1/2 activation induced by FGF2 in PC12 cells. As shown in FIG. 7D, overexpression of hIL-17RLM-L significantly suppressed the endogenous ERK phosphorylation, with the maximum inhibition at 10 min after stimulation. Additionally, hIL-17RLM exhibited an inhibitory effect on ERK activation in a dose dependent manner (FIG. 7E). These results indicated that

hIL-17RLM could inhibit FGF2-induced PC12 cell differentiation possibly through the inhibition of Ras-MAPK signaling.

Example 8 hIL-17RLM inhibited Ras-MAPK signaling pathway by acting on the up-stream signal molecule of Ras.

Next, we investigated which signaling component of Ras-MAPK pathway was suppressed by IL-17RLM. We recruited constitutively active Ras (G12V) or active MEK (MEK1RF) to check the activation of ERK by luciferase assay or Western blotting in PC12 cells. Both the luciferase assay and Western blotting analysis showed that hIL-17RLM had no effect on the signaling pathway mediated by constitutively active Ras (FIG 8A and B) and MEK (FIG 8C and D). These data were repeated in 293T cells (data not shown), suggesting that the target of IL-17RLM was probably located on the upstream signaling molecules of Ras in FGFR-Ras-MAPK signaling pathway.

Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. The preceding preferred specific embodiments are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever.

In the foregoing and in the examples, all temperatures are set forth uncorrected in degrees Celsius and, all parts and percentages are by weight, unless otherwise indicated.

The entire disclosure[s] of all applications, patents and publications, cited herein and of corresponding Chinese application No.02123447.7, filed June 28, 2002, is incorporated by reference herein.

The preceding examples can be repeated with similar success by substituting the generically or specifically described reactants and/or operating conditions of this invention for those used in the preceding examples.

From the foregoing description, one skilled in the art can easily ascertain the essential characteristics of this invention and, without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions.

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